Chapter 3

Growth factor-induced contraction of human bronchial smooth muscle is Rho-kinase dependent

Dedmer Schaafsma, Reinoud Gosens, Mechteld M. Grootte Bromhaar, Bart Vrught, Johan Zaagsma, Herman Meurs, S. Adriaan Nelemans

Abstract

Growth factors have been implicated in the pathophysiology of asthma. However, the putative effects of these growth factors on human airway smooth muscle tone are still largely unknown. We performed contraction experiments using human bronchial smooth muscle ring preparations. The growth factor insulin-like growth factor-1 (IGF-1) induced a slowly developing sustained contraction, which was dependent on Rho-kinase, since contraction was almost completely inhibited by (+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexane carboxamide (Y-27632; 1 μM). Angiotensin II, a Gq-coupled receptor agonist which can act as a growth factor as well, induced a biphasic contraction, the sustained phase of which was also almost completely inhibited by Y-27632. We conclude that angiotensin II and IGF-1 induce a Rho-kinase dependent sustained contraction of human bronchial smooth muscle. Since growth factors are associated with pathophysiological conditions such as asthma, inhibition of Rho-kinase could be effective under these conditions.

Introduction

Growth factors are involved in proliferation and differentiation of smooth muscle cells originating from a variety of tissues, including the vasculature and the airways [1,2]. They are potential contributors to the increased airway smooth muscle mass, as found in patients suffering from persistent severe asthma, by stimulating airway smooth muscle proliferation [2].

However, in vascular smooth muscle, several growth factors have been shown to induce a concentration-dependent contraction and to be potential inducers of contractile mediator release [3,4]. The mechanism by which growth factors induce contraction has only been partly elucidated. Recent evidence shows that growth factor receptors, such as the insulin-like growth factor-1 (IGF-1)-receptor, can activate the Rho/Rho-kinase pathway directly [5] and may be involved in smooth muscle contraction via Rho-kinase [6].

As regards airway smooth muscle, growth factors have been found to have long-term effects on bovine tracheal smooth muscle contractile phenotype [7] and acute contractile effects on guinea pig tracheal smooth muscle [8]. Thusfar however, the acute contractile effects of growth factors on human bronchial smooth muscle and the potential contribution of Rho-kinase has not been described. Therefore, we investigated human bronchial smooth muscle contraction induced by the growth factor IGF-1 and by angiotensin II which can transactivate different growth factor receptors [9] and was shown to be a hypertrophic growth factor for human airway smooth muscle cells [10]. In addition, we evaluated the possible involvement of Rho-kinase in growth factor-induced contraction.
Growth factor-induced human ASM contraction

**Methods**

Macroscopically normal sections of human lungs, obtained from patients undergoing surgery as a consequence of lung carcinoma, were transported to our laboratory in Krebs-Henseleit (KH) buffer (composition (mM): NaCl 117.5, KCl 5.60, MgSO\(_4\) 1.18, CaCl\(_2\) 2.50, NaH\(_2\)PO\(_4\) 1.28, NaHCO\(_3\) 25.00 and glucose 5.50, pregassed with O\(_2\):CO\(_2\) 95:5 % v/v, pH = 7.4). Human bronchi, typically 3-4 mm in diameter, were dissected free from connective tissue and cut into rings of approximately 3 mm in width. Bronchial rings were transferred into Dulbecco’s modification of Eagle’s medium, supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml) and maintained overnight at 37 °C in a humidified atmosphere containing 5 % CO\(_2\). The next day, bronchial rings were transferred into organ baths containing KH-buffer (37 °C), continuously gassed with O\(_2\):CO\(_2\) 95:5 % v/v. Rings were connected to an isometric force displacement transducer, using surgical wire. After equilibration for 90 min with 3 washings, rings were contracted using iso-osmotic KCl-solutions (20 and 40 mM) followed by washing. Finally, after re-equilibration in KH-buffer, rings were contracted with 40 mM KCl as a reference. After two additional washouts, rings were preincubated for 30 min with (+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexane carboxamide (Y-27632; 1 µM), a selective inhibitor of the Rho-kinase pathway [11] or vehicle, followed by the administration of growth factors or histamine.

**Data analysis**

All data represent means ± s.e.m. from n separate experiments. The statistical significance of differences between data was determined by one-way analysis of variance. Differences were considered to be statistically significant when P < 0.05.

**Materials**

Dulbecco’s modification of Eagle’s Medium was obtained from ICN Biomedicals (Costa Mesa, CA, U.S.A.). Penicillin/streptomycin solution (5000 U/ml ; 5000 µg/ml) was obtained from Gibco BRL Life Technologies (Paisley, U.K.). Insulin-like growth factor (IGF-1, human recombinant) and angiotensin II were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A). Y-27632 was from Tocris Cookson, Ltd. (Bristol, U.K.). All other chemicals were of analytical grade.

**Results**

IGF-1 (10 ng/ml) induced a slow rise of myogenic tone, reaching 17.3 ± 5.7 % of the 40 mM KCl response at t = 30 min (Figure 1a). This sustained contraction induced by IGF-1 was prevented almost completely in the presence of Y-27632 (1 µM, P<0.001; Figure 1a). In contrast to IGF-1, angiotensin II (1 µM) induced a biphasic contraction in time, typically reaching its first transient peak approximately at 2 min after administration, which averaged 18.9 ± 6.0 % of the KCl reference, followed by a slower and more sustained rise in myogenic tone, reaching a maximum of 24.5 ± 8.3 % at t = 10 min.
Interestingly, in the presence of the Rho-kinase inhibitor Y-27632 (1 μM), which lowered basal tone by itself, angiotensin II still induced the transient early-phase rise in contraction at \( t = 1 \) min (8.6 ± 3.0 %). The sustained rise, however, was no longer present \((P<0.001; \text{Figure 1b})\). Contractions induced by the \( G_q \)-coupled receptor agonist histamine were not affected at all by treatment with Y-27632 (Figure 2).

**Figure 1.** Time-dependent IGF-1 (a) and angiotensin II (b)-induced contraction of isolated human bronchi. Contractile responses were measured both in the absence (open symbols) and presence (closed symbols) of 1 μM Y-27632. Shown are the means ± s.e.m. of 3 to 4 experiments each performed in duplicate.

**Figure 2.** Histamine-induced contraction of isolated human bronchi. Contractile responses were measured both in the absence (open symbols) and presence (closed symbols) of 1 μM Y-27632. Shown are the means ± s.e.m. of 4 experiments each performed in duplicate.
Discussion

The results presented in this study demonstrate that the growth factors IGF-1 and angiotensin II induce Rho-kinase-dependent contractions of human bronchial smooth muscle. The concentrations of growth factor applied in this study are commonly used and represent submaximal to maximal concentrations with respect to other effects such as mitogenesis [12,13]. Quantitatively, these contractions amounted approximately 20-25% of 40 mM KCl-induced contraction. These limited contractile effects may clearly have physiological implications as small changes in internal diameter may result in significant limitation of airflow, since flow is proportional to internal radius to the fourth power (Poiseuille’s law).

Interestingly, histamine-induced contraction was not affected by this concentration of Y-27632, which indicates receptor-specificity for the contribution of Rho-kinase to contraction in the human bronchus. Similarly, methacholine- and KCl-induced contractions are differentially sensitive to Y-27632 as also observed in bovine tracheal smooth muscle [14][Chapter 6]. Since Y-27632 predominantly affected the sustained angiotensin II-induced contraction, it seems likely that angiotensin II receptors couple to the Rho/Rho-kinase-pathway selectively for this phase of contraction. In addition to the $G_\text{q}$-coupled consequences of angiotensin AT$_1$-receptor activation, the angiotensin AT$_1$-receptor has been shown to be able of transactivating different growth factor receptors [15]. Such a transactivation leads to activation of downstream signaling molecules also used by growth factors themselves. Our results show that Rho-kinase is not involved in the transient contraction by angiotensin II, but only in the slowly developing sustained phase, indicating possible transactivation of growth factor receptors by angiotensin II. In accordance with our observation that IGF-1-induced responses are fully Rho-kinase dependent, this phase is completely abolished by Y-27632.

Since growth factors are involved in tissue repair processes, growth factor-induced contraction may protect damaged areas in the airways from the outside air throughout the repair process. In asthma, however, the repair process is usually not restricted to a single segment of the airways and growth factors may then contribute to airflow obstruction. The growth factors used in the present study may be relevant for asthma for a number of reasons. The growth factor IGF-1 is known to be secreted in the airways from various inflammatory cells and airway smooth muscle [16]. In addition, plasma exudation may increase their tissue concentration during allergen exposure. Also, increased levels of the IGF-binding protein protease matrix metalloproteinase-1 (MMP-1) have been demonstrated in human asthmatic airways, which could increase the bioavailability of IGF-1 [17]. Angiotensin II has also been implicated in asthma as its serum levels are increased in patients with acute severe asthma [18]. Moreover, intravenous administration of angiotensin II in concentrations similar to those endogenously observed in severe asthmatics causes acute bronchoconstriction in mild asthmatics [19]. In addition, antigen-induced airflow hyperresponsiveness in guinea pigs has been found in part angiotensin AT1-receptor dependent [20].
Another Rho-kinase inhibitor, fasudil, is being tested in clinical trials and has been shown to be very effective in inhibiting symptoms associated with hypertension [21] and coronary artery spasm [22]. Rho-kinase inhibitors may surpass other drugs considering their selectivity for the pathophysiological condition. Such a pathophysiology-primed role may also be relevant in the airways, as repeated allergen challenge is known to increase the role of Rho-mediated Ca^{2+}-sensitization in antigen-induced airway hyper-responsive rats [23]. In addition, Y-27632 is known to suppress airway hyper-responsiveness induced by ovalbumin and respiratory syncytial virus in mice [24]. Therefore, the contraction induced by growth factors, which is completely dependent on Rho-kinase for IGF-1 and angiotensin II, may be more pronounced in inflamed areas and Rho-kinase inhibitors may relieve airflow limitations more pronouncedly in these areas when compared to control segments.

In conclusion, this study showed that the growth factors angiotensin II and IGF-1 induce a sustained contraction of human bronchial smooth muscle, which is completely dependent on Rho-kinase, in contrast to the histamine-induced contraction. Since growth factors are associated with pathophysiological conditions such as asthma, growth factor-induced contraction may be pathophysiologically relevant. Under these conditions, inhibition of Rho-kinase may be effective.

**Acknowledgements**

We would like to thank Mechteld M. Grootte Bromhaar and Annet Zuidhof for their expert technical assistance. This work was financially supported by the Netherlands Asthma Foundation, NAF grant 01.83.
References


